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Research note

Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles

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KEYWORDS

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Nanoparticles;
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Abstract Calcium alginate nanoparticles as excellent carriers in drug delivery systems were synthesized by controlled gellification method and characterized with Fourier Transform Infrared spectroscopy (FTIR). To compare mean particle size and distribution of calcium alginate nanoparticles with homopolymannuronate ones, later nanoparticles were prepared through the same conditions. Sodium homopolymannuronate is one of the ingredients of alginate polymer which was synthesized and purified by partial acid hydrolysis of sodium alginate and characterized with FTIR spectroscopy. Results showed significant improvement of size and distribution of calcium alginate nanoparticles with decrease of sodium alginate and increase of calcium cation concentrations, respectively. In addition, lower mean particle size and better distribution of calcium homopolymannuronate nanoparticles was observed in comparison with calcium alginate ones. This result may refer to the ionic interaction of calcium crosslinker ions with regular homopolymeric chains of homopolymannuronate compared to no regular chains of alginate polymer.

1. Introduction

Polymeric nanoparticles have been in focus in recent years because of their clinical usages for diagnostics, therapeutics and carriers for delivery systems [1–3]. Different methodologies have been reported including interfacial polymerization, solvent evaporation, solvent deposition, nanoprecipitation, emulsification-diffusion and controlled gellification for synthesizing of nanoparticles [4–6]. Among these methods, controlled gellification technique is very susceptible to concentration of the ingredients of nanoparticles and therefore special proficiency is necessary to obtain particles with nano-sized dimensions [7].

Alginic acid and its derivatives as biopolymers have been attracted more attention due to their unique properties during recent years [8,9]. These polymers have two main sources of bacteria and brown algae. Alginates with algae's sources show different structural and chemical properties with respect to their seasonal and growth conditions. These polymers can be described as linear binary copolymers of 1–4-linked M and G residues arranged with homopolymeric regions of α-L-guluronic acid residues (G-blocks) and a homopolymeric region of β-D-mannuronic acid sequences (M-blocks) interspersed by regions in which the two groups coexist in a strictly alternating sequence (MG-blocks) (Figure 1). The saccharide units of the alginate chain i.e. mannuronic acid and guluronic acid residues accept different conformations. It follows that diequatorial linkages connecting mannuronic acid residues in M-blocks can be assumed as a flat ribbon-like chain conformation. In contrast, diaxially-linked guluronic acid residues lead to a more rigid structure for the G-blocks. MG-blocks are characterized by alternating axial–equatorial and equatorial–axial glycosidic bonds connecting the residues [10]. It has been reported that the rigidity of the chain blocks was decreased along the series GG > MM > MG [11]. Alginic acid and its carboxylic salts are biopolymers which show interesting features such as biocompatibility, biodegradability, viscosifying and the ability of gelation with multivalent cations [12]. In addition, these polymers have been introduced as encapsulation agents and therefore have found different applications in drug delivery

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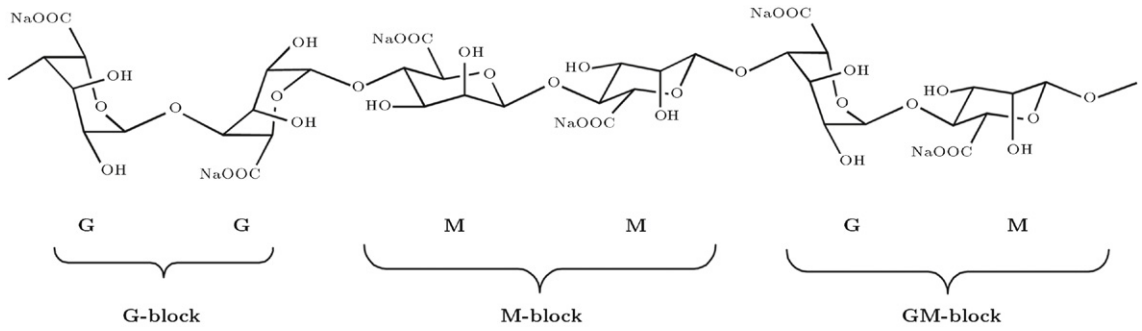

Figure 1: Chemical structure of sodium alginate.

and control release systems. The property of crosslinking of alginate's salts such as sodium alginate by divalent calcium cations afforded that calcium alginate gels have been known as excellent carriers for a variety of drugs in drug delivery systems [13,14]. Calcium alginate nanoparticles with mean particle size 400–500 nm have recently been synthesized and argued as the novel encapsulation agents for drugs [15]. On the other hand, homopolymannuronate ingredient as the regular chains of the alginate with diequatorial linkages between mannuronic acid residues is synthesized by acid hydrolysis of alginate [16,17]. To the best of our knowledge, there is no report in literature concerning the preparation of calcium homopolymannuronate nanoparticles. The aim of this research is the preparation and study of calcium alginate nanoparticles with mean particle size under 100 nm and following comparison with calcium polymannuronate ones. On the other hand, the nanoparticles with mean particle size under 100 nm due to their more surface area compared to previous reports should show significant interactions with drugs and better results in drug delivery systems.

2. Material and methods

2.1. Materials

Sodium Alginate (SA) with number-averaged molecular weight 12,000–40,000 was purchased from Sigma and used as received. Calcium chloride ($CaCl_2$) and hydrochloric acid (HCl) were supplied from Merck Chemicals Co. and were used as the crosslinker and acidic catalyst for hydrolysis of sodium alginate, respectively. Deionized water was obtained from IPPI. All the reagents used in this research were obtained as analytical grade.

2.2. Partial acid hydrolysis of alginate

Sodium alginate was partially hydrolyzed according to the controlled gellification method [16]. One gram of polysaccharide was dissolved in 100 mL of deionized water at 50 °C and heated at reflux with 3 mL of HCl 3 M for 20 min. After cooling, the suspension was centrifuged (3000 × g, 20 min) and the insoluble fraction from the centrifugation was refluxed with 100 mL of HCl 0.3 M during 2 h. After centrifugation (8500 × g, 20 min), the insoluble material was neutralized (NaOH 1 M) and the pH was adjusted to 2.85 with HCl 1 M. The soluble fraction was neutralized and added to 100 ml of ethanol. The precipitate was collected by centrifugation (8500 × g, 20 min) and was dried at 50 °C invacuo for 12 h (block M).

2.3. Synthesis of calcium alginate nanoparticles and calcium homopolymannuronate ones

In order to study the effective parameters on the size and distribution of calcium alginate nanoparticles, solutions of SA with different concentrations (0.03%, 0.06%, 0.12% w/v) were obtained by dissolving proper amounts of polymer in deionized water at room temperature. Then the required amounts of $CaCl_2$ were dissolved in deionized water to obtain clear solutions with precise concentration (18, 36 mM). After homogenization of sodium alginate solution by mechanical stirrer, the solution of calcium chloride was added to the system. After 1 h of the rotation, prepared nanoparticles were purified by ultracentrifugation for 20 min. To compare calcium alginate nanoparticles with calcium homopolymannuronate ones, later nanoparticles were prepared through the same conditions. The concentrations of sodium homopolymannuronate and $CaCl_2$ solutions for preparation of calcium homopolymannuronate nanoparticles were 0.03% w/v and 18 mM, respectively.

2.4. Measurements

The IR spectra of the sodium alginate, sodium polymannuronate and calcium alginate nanoparticles were performed with a Bruker-Equinox 55 FTIR spectrometer (Ettlingen, Germany) which was equipped by H.ATR accessories with a ZnSe crystal. Mean particle size and distribution of prepared nanoparticles were studied by Scanning Electron Microscopy (SEM) (VEGA, TESCAN).

3. Results and discussion

Nanoparticles of calcium alginate were obtained by addition of $CaCl_2$ to solution containing sodium alginate by mechanical stirrer at high stirring rates. In order to compare the size and distribution of calcium polymannuronate nanoparticles with its initial source, i.e. alginate, nanoparticles of calcium alginate were prepared by the controlled gellification. Sodium homopolymannuronate as one of the ingredients of alginate was prepared by partial acidic hydrolysis of alginate and characterized by IR spectroscopy.

3.1. IR spectroscopy

The Fourier transform infrared (FTIR) spectra of the sodium alginate, sodium polymannuronate and calcium alginate nanoparticles were recorded and compared (Figure 2). Spectrum of sodium alginate (Figure 2(a)) showed important absorption bands regarding hydroxyl, ether and carboxylic

functional groups. Stretching vibrations of O–H bonds of alginate appeared in the range of 3000–3600 cm^{-1}. Stretching vibrations of aliphatic C–H were observed at 2920–2850 cm^{-1}. Observed bands in 1649 and 1460 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of carboxylate salt ion, respectively. Later bands are very significant and can be used for characterization of alginate structure from its derivatives and ingredients. The bands at 1107 and 935 cm^{-1} were attributed to the C–O stretching vibration of pyranosyl ring and the C–O stretching with contributions from C–C–H and C–O–H deformation. On the other hand, sodium polymannuronate which was obtained by partial acid hydrolysis of alginate showed some different bands, especially at anomeric region compared to alginate spectrum (Figure 2(b)). It is remarkable that the asymmetric stretching vibration of the carboxylate group appears at 1620–1598 cm^{-1}. The shift to lower wave numbers in comparison with the sodium alginate samples may indicate an interaction of the regular homopolymeric chain with the sodium ions. The main peak in the IR spectra of homopolymannuronate appeared at 1100–1010 cm^{-1} which was attributed to the C–O stretching vibration of pyranosyl ring and the C–O stretching vibrations. In the fingerprint region, the homopolymannuronate sample showed a band at 935 cm^{-1} assigned to the C–O stretching vibration of uronic acids with contributions from C–C–H and C–O–H deformation, and a band at 885 cm^{-1} assigned to deformation vibration of β-C_1–H. Calcium alginate nanoparticles powder showed significant differences bands in comparison with IR spectrum of SA (Figure 2(c)). Absorption region of stretching vibrations of O–H bonds in calcium alginate appeared narrower than SA. This difference arises from the participation of hydroxyl and carboxylate groups of alginate to the calcium ion in order to form chelating structure and consequent decrease in hydrogen bonding between hydroxyl functional groups which affords narrower band in calcium alginate. Asymmetric stretching vibration of carboxylate ion shifted to lower wave numbers because when calcium metal ions replaced sodium ions in the sodium alginate, the charge density, the radius and the atomic weight of the cation were changed and hence, this shifting should be expected. It is obvious that the bands concerning carboxylate groups can be used as useful bands to follow the changes in the structure of different polymers of the alginate.

3.2. Effect of polyanionic alginate and calcium cation concentrations

It has been reported that concentrations of calcium crosslinker cations and polyanionic alginate have important effects on the mean particle size and morphology of nanoparticles. For probing the effect of alginate concentration, SA solutions with different concentrations (0.03%, 0.06% and 0.12% w/v) were prepared by dissolving proper amounts of sodium alginate in deionized water. Then nanoparticles were obtained by addition of $CaCl_2$ solution (18 mM) into the SA solutions at high stirring rates of mechanical stirrer. The Prepared samples were purified by centrifugation (8500 × g, 10 min) and spread over a glass lamella and dried under vacuum at 70 °C before SEM analysis. It is apparent from SEM images that the mean particle size and distribution of nanoparticles improve with decreasing of SA concentration (Figure 3) and (Table 1). This improvement in mean particle size was related to the decrease of alginate concentration and subsequent reduction in sodium alginate chains and number of the carboxylate, ether and hydroxyl groups that are jointed to the calcium cation. It has been reported that the main factor for creating nanoparticles is the tendency of functional groups of alginate chains especially carboxylate groups to create complex structures with calcium ions. The increase of alginate concentration affording more functional groups can gather around calcium crosslinking agent and therefore further layers of alginate chains can join the calcium cations; in conclusion, the size of nanoparticles increases with increasing of alginate concentration.

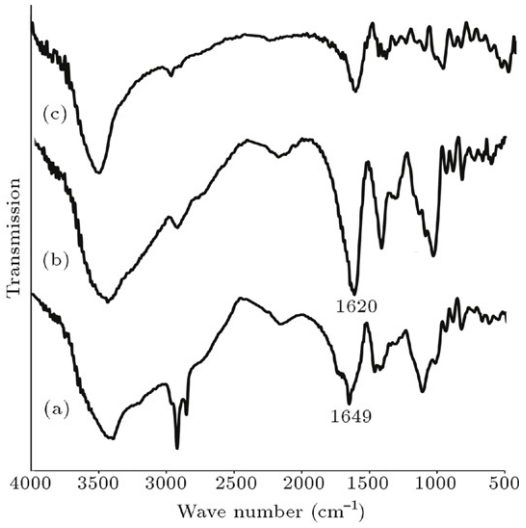

Figure 2: FTIR spectra of the polymers. (a) Sodium alginate; (b) sodium homopolymannuronate; and (c) nanoparticles of calcium alginate.

Table 1: Effect of concentration of sodium alginate solution on dimension and properties of nanoparticles.

Concentration of sodium alginate (mM)	0.03%	0.06%	0.12%
Dimension of nanoparticles[a] (nm)	50	70	>1000
Distribution	Excellent	Very good	–
Dispersion	Excellent	Very good	–

[a] Concentration of calcium solution was 18 mM.

Table 2: Effect of the concentration of calcium chloride solution on dimension and properties of nanoparticles.

Concentration of calcium cation (mM)	18	36
Dimension of nanoparticles[a] (nm)	60–70	40–50
Distribution	Very good	Excellent
Dispersion	Very good	Excellent

[a] Concentration of alginate solution was 0.06% w/v.

In addition of the alginate, the concentration of calcium ions is an important parameter to obtain nanoparticles of calcium alginate. The examination of the effects of calcium ion on the particle's size was done by the addition of $CaCl_2$ solution with different concentration (18, 36 mM) to the SA solution (0.03% w/v). Based on predictions, with increasing calcium ion concentration, lower numbers of the polymer chains are involved with higher contents of calcium cations and therefore the size of nanoparticles must be smaller clearly (Table 2). SEM images of prepared samples showed that the mean particle size and distribution of nanoparticles significantly improved due to higher contents of calcium ions (Figure 4).

To compare calcium alginate nanoparticles with calcium homopolymannuronate ones, later nanoparticles were prepared by the addition of calcium chloride (18 mM) into a homopolymannuronate solution (0.03% w/v) at high rates of mechanical

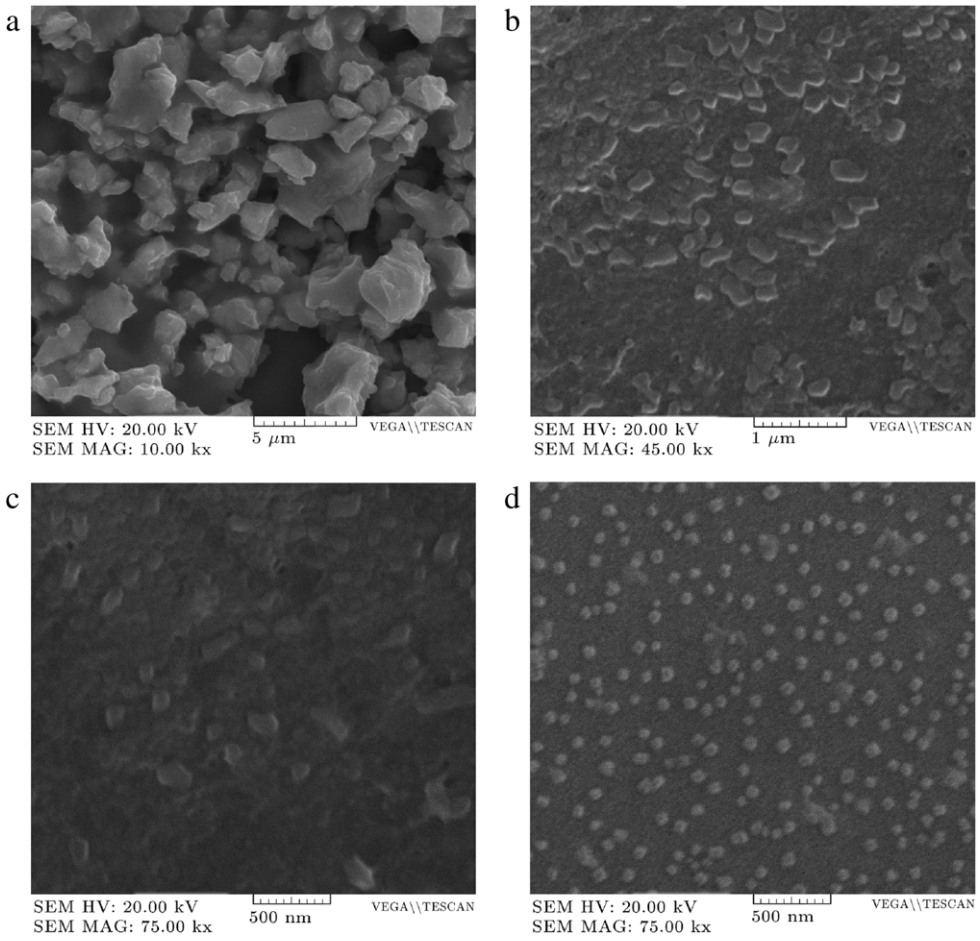

Figure 3: SEM images of nanoparticles of calcium alginate obtained by different concentrations of sodium alginate salt. (a) 0.12% w/v; (b) and (c) 0.06% w/v and (d) 0.03% w/v.

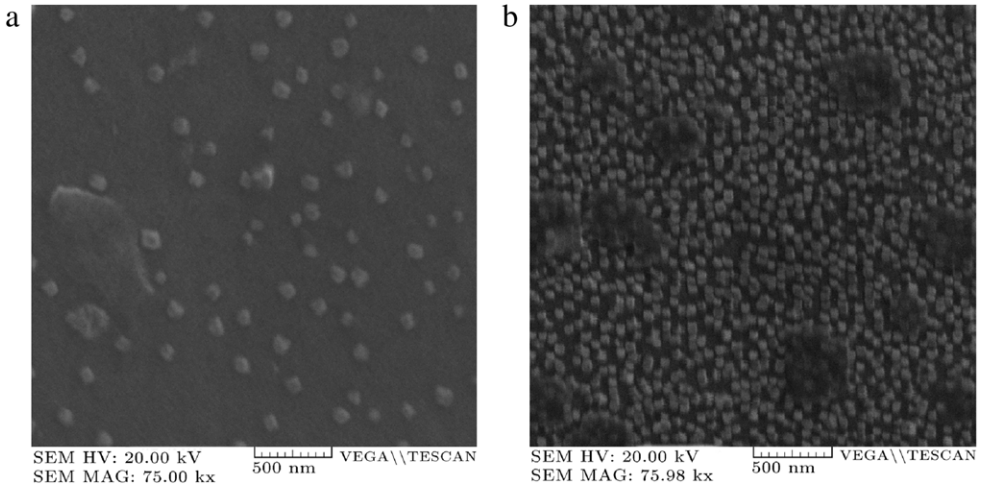

Figure 4: SEM images of nanoparticles of calcium alginate obtained by different concentrations of calcium chloride cross-linker. (a) 18 mM; and (b) 36 mM.

stirrer. Dimensions of both homopolymannuronate and alginate particles showed satisfactory success to obtain nanoparticles with sizes under 100 nm in comparison with previous works by other researchers (Figure 5). In addition, distribution and dispersion of prepared nanoparticles showed more remarkable results than previous reports. The mean particle size and distribution of calcium homopolymannuronate showed significant improvement in relation to similar calcium alginate

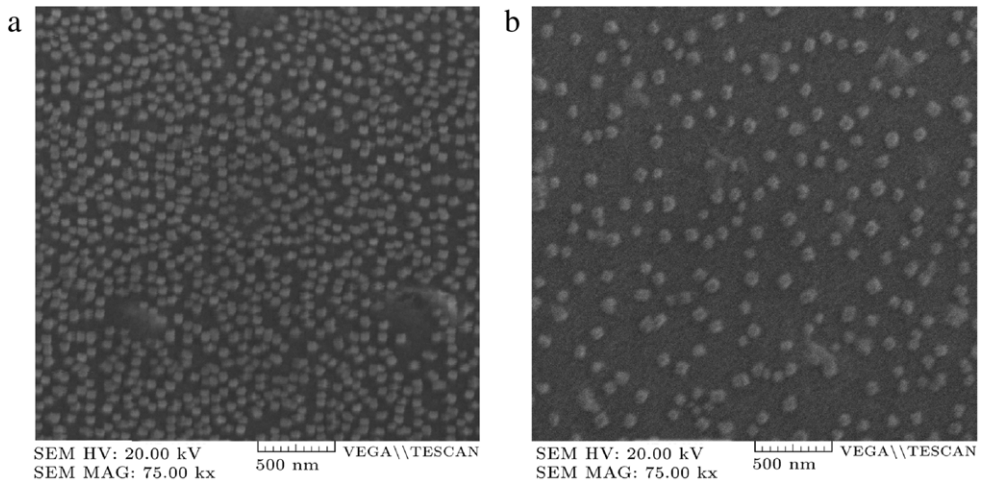

Figure 5: SEM images of (a) calcium homopolymannuronate nanoparticles, and (b) calcium alginate nanoparticles.

nanoparticles. This improvement may relate to the interaction of calcium ions with the regular homopolymeric chain of homopolymannuronate, while this regularity is not seen in alginate chain structure.

4. Conclusion

Nanoparticles of calcium alginate were synthesized by the addition of calcium chloride solution to the dilute solution of sodium alginate by controlled gellification method. Due to the random distribution of diequatorial and diaxial linkages, alginate chains have different conformations that afford multivalent cations showing different interactions with each block. Sodium homopolymannuronate was prepared by partial acid hydrolysis of sodium alginate, purified by ethanol and characterized with IR spectroscopy. Diequatorial linkages that connect mannuronic acid residues together in M-blocks of alginate cause a flat ribbon-like chain conformation. This conformation affords the formation of complex structures by multivalent cations because of the interaction of these cations and mentioned regular chain structures. Nanoparticles of calcium homopolymannuronate were synthesized by controlled gellification method in this research. The comparison of SEM images of calcium homopolymannuronate nanoparticles with nanoparticles of alginate polymer has revealed that regular chains of homopolymannuronate are able to form better coordination structure with calcium ions, therefore calcium homopolymannuronate nanoparticles showed better distribution and smaller particles in comparison with alginate nanoparticles. It is possible to examine these nanoparticles as proper carriers in drug delivery systems because of their nano-sized structures and high surface area. It is important to note that this examination is under progress and more results of drug delivery of prepared nanoparticles will be reported in future publications.

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